

*On the Occurrence of Gelatinous Spicules, and their Mode of Origin, in a New Genus of Siliceous Sponges.*

By ARTHUR DENDY, D.Sc., F.R.S., Professor of Zoology in the University of London (King's College).

(Received March 25, 1916.)

[PLATE 11.]

The siliceous microscleres, or flesh-spicules, of the tetraxonid sponges have long been regarded by those who have studied them as amongst the most beautiful and at the same time most inexplicable structural phenomena met with in the organic world. Their exquisite symmetry, their great diversity in form in different genera and species, and their remarkable constancy in details of shape within the limits of the same species, taken in conjunction with the fact that it is impossible to account for this shape by reference to any function that they may perform in the vital economy of the sponge, constitute a problem of no little interest to the philosophical biologist.

These spicules are generally stated to be composed of hydrated silica, or opal. So far as has been known hitherto, they are perfectly transparent, hard and brittle, and are unaffected by prolonged boiling with strong acids. They are universally believed to be intracellular in origin, and on several occasions have been figured within nucleated mother-cells. The evidence on this point is, however, rather scanty and by no means conclusive, and though several observers have studied the mature form of these spicules in great detail, singularly little is actually known about their mode of origin.

The chief object of the present communication is to describe an entirely new type of spicule, differing, not so much in form as in chemical composition, from any previously known, the study of which, it is hoped, may throw considerable light on the nature and origin of siliceous microscleres in general.

The most striking and novel feature about the spicules in question is that, although still composed, so far as can be ascertained, of colloidal silica, they are gelatinous, contracting greatly in alcohol and swelling up again on addition of water. I first observed them in Australia many years ago, when engaged in cataloguing the great collection of Victorian sponges made by the late Mr. Bracebridge Wilson. They were found in only a single specimen, and their nature was so problematical and the sponge in which they occurred so ill-characterised in other respects that I set it aside as "indeterminable at present," and did not include it in my published catalogue.

Recently, while investigating and reporting upon a large collection of sponges from the Indian Ocean, made by the "Sealark" Expedition under the leadership of Prof. Stanley Gardiner, I have come across three more specimens containing similar spicules, and have been led to make a detailed examination of these enigmatical bodies. The "Sealark" specimens belong to a perfectly distinct species, but probably related not distantly to the Australian sponge. I propose for the reception of the latter the new genus *Collosclerophora*. A diagnosis of this genus and a brief description of the type species, *Collosclerophora arenacea*, are given at the end of the present paper. I reserve an account of the Indian Ocean sponge for my report on the "Sealark" collection.

Unfortunately the amount of material at my disposal for the investigation of the jelly-spicules of *Collosclerophora arenacea* was extremely small. There remained in my possession only a rough, unstained Canada balsam mount, consisting of a single thick, hand-cut section prepared more than twenty years ago.

This preparation showed the jelly-spicules or "colloscleres," as I propose to term them, with great distinctness and in very large numbers, scattered through the soft tissues. They varied considerably in shape, as shown in Plate 11, fig. 1, some being sausage-shaped, others boomerang-shaped, and others again kidney-shaped, but always with a more or less distinct notch or indentation on one side. The length of these bodies, measured in a straight line from end to end, varied from about 0.02 to 0.03 mm. Although they had perfectly sharp outlines, they were somewhat less bright in appearance than ordinary microsccleres, and it was at once evident that there was, apart altogether from their shape, something peculiar about them.

In order further to investigate the nature of the colloscleres it was necessary to remove the section from the slide on which it was mounted. This was effected by soaking in xylol, the Canada balsam being thus completely extracted. Part of the section was remounted in balsam, and the remainder transferred to alcohol.

After soaking in xylol the colloscleres almost completely disappeared from view, so that in the fragments remounted in xylol balsam they were barely recognisable, though the ordinary spicules were of course brilliant, there being a great difference in refractive index between the two. I found them little, if any, more distinct in chloroform balsam, and it would appear as if very prolonged soaking in balsam were necessary to render them as distinct as they were in the original preparation. They are not really soluble either in xylol or chloroform.

The original section unfortunately consisted chiefly of sand-grains, which

had to be picked out one by one with needles in order to make preparations suitable for more minute examination, but sufficient of the sponge-tissue remained to enable me to make the following observations.

When mounted in a drop of absolute alcohol the colloscleres are perfectly distinct and may sometimes be seen to be enclosed each in a thin-walled vesicle (fig. 2). When a drop of water is run in under the cover-glass of such a preparation the colloscleres suddenly swell up to several times their previous volume and become extremely transparent (fig. 2*a*; fig. 3, *b*, *c*). The swelling takes place chiefly on the convex side, which becomes more strongly convex, while the line of demarcation between it and the surrounding water becomes almost invisible. The contour of the concave side, on the other hand, is quite bright and distinct, and usually distinctly double. I conclude from this that the substance of which the collosclere is composed is denser and less absorbent of water on the concave than on the convex side. I also conclude that in life these spicules, if spicules they can be termed, exist in the sponge in the swollen, gelatinous condition, and that the contracted state first observed is due simply to the withdrawal of water by means of alcohol. It seems remarkable that they should retain their power of absorbing water and swelling up even after more than twenty years in Canada balsam.

In a very strong cold solution of caustic potash the colloscleres, already swollen in water, swell up further and then dissolve.

When a 5 per cent. solution of hydrochloric acid is run in under the cover-glass they swell up and become invisible in the surrounding tissues, but there is no effervescence and I do not think they really dissolve. At any rate, the contour of the concave surface may remain distinctly visible even after prolonged action of strong hydrochloric acid (fig. 3, *b'*), and a preparation stained with paracarmine and mounted in Canada balsam after treatment with dilute hydrochloric acid shows at least one collosclere quite distinctly.

The colloscleres stain quite readily with paracarmine, but with a solution of iodine in potassium iodide I obtained only negative results. When examined with the polariscope they exhibit no optical activity either when contracted in alcohol or when swollen in water. From these observations I think it may be concluded with a reasonable degree of certainty that the colloscleres are composed of colloidal silica containing a much higher percentage of water than the ordinary siliceous spicules of the same sponge.

Teased preparations, stained with paracarmine and mounted in Canada balsam after removal of the sand-grains, throw a great deal of light on the origin of the colloscleres. They are found to be associated in the mesogloea with large spherical cells, which, for reasons which will appear directly,

I regard as their mother-cells or scleroblasts (silicoblasts). These scleroblasts occur in groups, sometimes so closely packed together as to become polygonal from mutual pressure (fig. 4), sometimes more loosely arranged (figs. 5-8). Each one is about 0.02 mm. in diameter and provided with a very thin cell-membrane. When darkly stained (figs. 5 and 6) the whole cell appears rather coarsely granular and no nucleus is visible. When more lightly stained (fig. 4) it is seen that the cell is vesicular and contains a small nucleus, apparently suspended in the middle by threads of cytoplasm, while the highly refringent granules lie against the inner surface of the cell-membrane. Except for the presence of these granules the scleroblasts agree very closely with the well-known form of sponge-tissue termed by Sollas cystenchyme.

Amongst the scleroblasts lie the colloscleres; each is commonly enclosed in an oval, thin-walled vesicle very much larger than itself (figs. 5, 6, 8), though it seems probable that in life it may have completely filled the vesicle. The wall of the vesicle stains very distinctly and in much the same way as does the collosclere itself.

It is by no means easy to determine the exact relations between the colloscleres, their vesicles, and the scleroblasts. In teased preparations the vesicles, with their contained colloscleres, sometimes appear quite separate and isolated, but there is, I think, no question of the vesicle itself being the wall of a mother-cell. Not infrequently one vesicle can clearly be seen attached sideways to a single scleroblast, as shown in figs. 9 and 10, and in such cases it is hard to resist the conviction that the vesicle, or, at any rate, its contents, is in some way or other the product of the scleroblast. In such cases the collosclere usually lies at the side of the vesicle remote from the scleroblast, with its concave surface turned towards the latter.

In other cases a vesicle may appear to be attached end-on to a scleroblast, as shown in fig. 8, but I am not satisfied that the association in such cases is not accidental, and that the scleroblasts that really belong to the vesicles in question have not been removed in the course of preparation. In fig. 11 a vesicle is shown which has quite evidently been torn partially away from a scleroblast, but whether its own or not it is again impossible to say.

The strongest evidence that the colloscleres really are secreted by the cells which I have ventured, perhaps somewhat prematurely, to call the scleroblasts, is as follows. A large proportion of these cells are found to exhibit a well-defined rounded knob, attached to the outer surface of the cell membrane at one pole, as shown in fig. 3, *a*, and fig. 7. In fig. 7 the four cells represented do not seem to have been disarranged in the course of preparation, and each one shows the characteristic knob, all the knobs, curiously enough, pointing in approximately the same direction. These

knobs offer a striking contrast to the cells themselves in their hyaline, non-granular character. They stain fairly darkly with paracarmine and appear to be entirely devoid of structure. They are attached to the scleroblasts by broad bases and vary considerably in size, progressive stages of growth being represented in figs. 12-16. They evidently represent an extracellular secretion of the scleroblasts, possibly derived from the minute refractive granules which line the inner surface of the cell-membrane. The largest knobs are of just about the same size as the contracted colloscleres, but, unlike the latter, they do not lie in hollow vesicles and do not swell up on addition of water. This fact seems, at first sight, to negative the view that the knobs are really the colloscleres in process of secretion, but I think the apparent discrepancy may possibly be explained as follows.

We have already seen that the colloscleres always exhibit an indentation or notch on one side, which we may conveniently term the hilum. This may be taken to represent the original attachment of the collosclere to the scleroblast, an attachment which is strongly suggested by the appearance represented in fig. 9. We have also seen that, on the addition of water, the contracted collosclere swells up chiefly on the convex side, the concave surface, which is supposed to lie next to the scleroblast, being apparently formed of much denser silica. We are therefore, I think, justified in assuming, at any rate provisionally, that the secretion, when first discharged from the scleroblast, is in a concentrated condition, and that it only acquires the property of absorbing water and swelling up after the lapse of a longer or shorter interval. It seems not unlikely that the swelling up may be coincident with its complete separation from the scleroblast.

Probably, in life, as I have already suggested, the swollen collosclere completely fills the vesicle in which it lies, and the wall of the vesicle may be regarded either as a concentration of the mesogloea due to the pressure of the collosclere, or as a precipitation membrane formed at the surface of contact between the gelatinous collosclere on the one hand and the gelatinous mesogloea on the other, the former consisting of colloidal silica and the latter presumably of an albuminoid character.\*

It appears, then, that the colloscleres are gelatinous spicules of colloidal silica, formed by special scleroblasts or mother-cells, but as extracellular and not intracellular secretions. They are undoubtedly a normal constituent of the sponges in which they have been found, and, as I have already pointed out, they occur in two perfectly distinct, though related species. In the Indian Ocean species they are much smaller than in the Australian, and are associated with smaller scleroblasts. I have not traced their development

\* Probably both contain small quantities of mineral salts.

in this species, but I have been able to demonstrate with the greatest ease that they swell up on the addition of water just as they do in the Australian sponge.

In the Indian Ocean species they are also associated with large numbers of minute palmate isochelæ of the usual *Clathria* type. Indeed, until I discovered their property of absorbing water and swelling up, I had no doubt that the colloscleres in this sponge were merely modifications of these isochelæ, with the space between the shaft and palms filled up with silica, a view which was strongly supported by the occurrence of what appear to be intermediate forms.

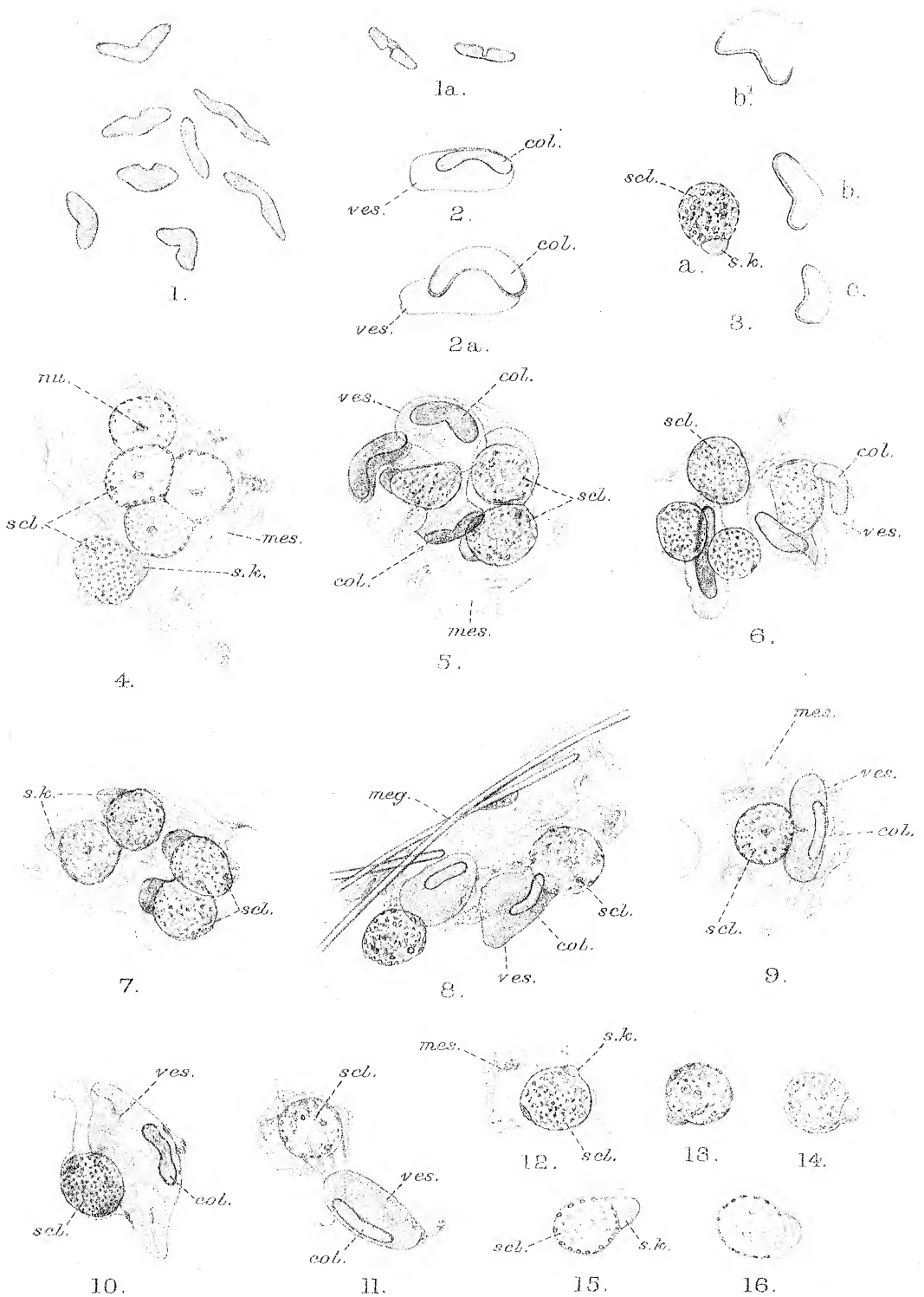
In *Collosclerophora arenacea* I have seen only one or two isochelæ and it is doubtful if such spicules any longer form a normal constituent of the very degenerate spiculation, though here again one occasionally sees what look like intermediate forms between colloscleres and isochelæ (fig. 1a).

In view of the generally accepted ideas as to the intracellular origin, not only of chelæ, but of microscleres in general, it would no doubt be premature to insist upon the homology of the colloscleres with isochelæ. Such ideas, however, are not based upon a very firm foundation. It seems not impossible that what have hitherto been taken to be the nuclei of mother-cells are really, at any rate in the case of the chelæ, entire scleroblasts adhering to the spicules, while the supposed membrane of the mother-cell is the wall of an extracellular spicule-vesicle. Further investigations into the origin of siliceous microscleres are urgently called for, and until these have been carried out it is inadvisable to propound any general theory on the subject. The occurrence of gelatinous spicules is in itself, however, such a remarkable and unique phenomenon that it seems desirable to place it on record without further delay.

#### Genus COLLOSCLEROPHORA n. gen.

*Diagnosis.*—Tetraxonid sponges with gelatinous microscleres (colloscleres). The normal skeleton is almost entirely replaced by sand-grains. The megascleres are slender strongyla.

The type-species of the genus (*Collosclerophora arenacea*) is a typical sand-sponge with much reduced spiculation, and were that species alone available for study it would hardly be possible to form a definite conclusion as to its proper position in the tetraxonid series. Fortunately the Indian Ocean species, which will be described in my Report on the Sponges of the "Sealark" Expedition, still preserves a full complement of both mega- and microscleres, which enables me to refer it without hesitation to the



Ectyoninæ, and to infer that the Australian species probably belongs to the same sub-family, though perhaps generically distinct.

*Collosclerophora arenacea* n. sp.

Sponge massive, sessile, solid, with evenly rounded convex upper surface showing parallel sandy tracts, separated by intervening areas with minutely reticulate dermal membrane. Vents small, scattered in intervening areas. Texture incompressible, friable, intensely sandy, the sand being arranged in a lamino-reticulate fashion.

*Megascleres*.—Slender strongyla, straight or nearly so, measuring about 0.22 by 0.0025 mm., smooth and with evenly rounded ends. These spicules are very numerous, occurring chiefly in loose wisps radiating towards the surface, where they form sparse surface-brushes. There is no other dermal skeleton.

*Microscleres* (figs. 1, 1*a*).—Colloscleres of varying form. When contracted, always with an indentation or notch on one side; sausage-shaped, boomerang-shaped and kidney-shaped. These spicules swell up and become gelatinous on addition of water (figs. 2*a*; 3, *b*, *c*).

The single specimen was dredged by Mr. J. Bracebridge Wilson in the summer of 1888–9 at Station 1, near Port Phillip Heads, and is entered as R.N. 923 in my manuscript catalogue, from which the above details as to external characters are taken.

DESCRIPTION OF PLATE 11.

*Collosclerophora arenacea* n. gen. et sp.

Fig. 1.—Colloscleres in the contracted state, as seen in an old Canada balsam preparation, unstained. ×460.

Fig. 1*a*.—Two contracted colloscleres from the same preparation, approaching isochelæ in form. ×460.

Fig. 2.—A collosclere enclosed in its vesicle, separated in absolute alcohol. ×480.

Fig. 2*a*.—The same collosclere after swelling up in water. ×480.

Fig. 3.—A scleroblast (*a*) and two colloscleres (*b* and *c*) examined in water, unstained. The colloscleres have swollen but the secretion-knob on the scleroblast has not; *b'*, the same collosclere as represented in *b*, after running in a drop of fuming hydrochloric acid from the edge of the cover-glass. ×480.

Fig. 4.—A group of scleroblasts surrounded by mesogleæ; from a teased preparation, very lightly stained with paracarmin and mounted in Canada balsam. The four upper cells show the nucleus very distinctly; the lowest one is focussed on the cell-membrane and shows a secretion-knob. ×480.

Fig. 5.—A group of scleroblasts in the mesogleæ, with associated colloscleres and vesicles. From a teased preparation stained with paracarmin and mounted in Canada balsam. ×480.



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- Fig. 6.—Another group of scleroblasts with associated colloscleres and vesicles; under the same conditions as fig. 5.  $\times 480$ .
- Fig. 7.—A group of four scleroblasts lying undisturbed in the mesogloea and each showing a secretion-knob. Stained with paracarmine and mounted in Canada balsam.  $\times 480$ .
- Fig. 8.—Two scleroblasts with associated colloscleres and vesicles, lying undisturbed in the mesogloea with portions of three megascleres. Stained with paracarmine and mounted in Canada balsam.  $\times 480$ .
- Fig. 9.—A single scleroblast with associated vesicle and collosclere probably secreted by itself, lying undisturbed in the mesogloea. Stained with paracarmine and mounted in Canada balsam.  $\times 480$ .
- Fig. 10.—Another scleroblast, isolated by teasing, with its associated vesicle and collosclere. Stained with paracarmine and mounted in Canada balsam.  $\times 480$ .
- Fig. 11.—A vesicle, with contained collosclere, partially torn away from a scleroblast by teasing. Stained with paracarmine and mounted in Canada balsam.  $\times 480$ .
- Figs. 12–16.—Five scleroblasts, showing stages in the growth of the secretion-knob. From teased preparations stained with paracarmine and mounted in Canada balsam.  $\times 480$ .

*Explanation of Lettering.*—*col.*, collosclere; *meg.*, megasclere; *mes.*, mesogloea; *nu.*, nucleus; *scl.*, scleroblast; *s.k.*, secretion-knob; *ves.*, vesicle containing collosclere.

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## *On the Experimental Production of Congenital Goitre.*

By ROBERT MCCARRISON, M.D., D.Sc., F.R.C.P. (lately on Special Duty  
for the Study of Goitre and Cretinism in India).

(Communicated by Sir Victor Horsley, F.R.S. Received March 18, 1916.)

### [PLATE 12.]

The experiment herein described was undertaken at the Central Research Institute, Kasauli, India. It commenced on September 6, 1913, and terminated, owing to my recall to military duty for active service, on December 24, 1914. Having been on service for the past 18 months I have not hitherto had an opportunity to report it.

### *Object of the Experiment.*

Its object was to determine the cause of congenital goitre and the conditions under which it developed in large animals, and to confirm and amplify the results I had obtained by previous experimentation on white rats.

It was consequently designed so as to subject the fœtuses of primiparæ to

